Discriminant Model for Cytologic Distinction of Large Cell Neuroendocrine Carcinoma from Small Cell Carcinoma of the Lung

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Background: To establish cytologic criteria for pulmonary large cell neuroendocrine carcinoma (LCNEC), we developed and evaluated a discriminant model for cytologic differential diagnosis between LCNEC and small cell lung carcinoma (SCLC).

Methods: Aspiration cytologic and/or imprint smears from 29 LCNEC cases were reviewed in comparison with 26 SCLC cases. We selected the following parameters for assessment: background, cellular arrangement, cell clusters, cell cohesion, arrangements, cell dimensions areas, the presence of cytoplasm and/or prominent nucleoli, nuclear features, mitosis, naked nuclei, and nuclear streaking. To demonstrate the utility of differences in frequencies of cytologic parameters for LCNECs and SCLCs, a discriminant model was developed and evaluated.

Results: Among the cytologic parameters investigated, large clusters (consisting of $\geq$60 tumor cells) with tight cohesion and small tumor cells (showing $\leq$120 μm^2) without prominent nucleoli on each case were particular focuses of attention, because statistically significant differences with good power were evident between the LCNEC and SCLC groups for their frequencies ($p < 0.0001$). On the basis of variation in plotted location on scatter plots, a discriminant model for LCNEC and SCLC was made and evaluated by logistic discriminant analysis. Sensitivity, specificity, and accuracy were all 100%. With leave-one-out cross validation, the predicted error rate of the discriminant model for new cases was 0.00545.

Conclusion: Our model based on the cytologic features of large cell clusters with tight cohesion and of small tumor cells without prominent nucleoli should be a useful aid for distinction between LCNECs and SCLCs.

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Large cell neuroendocrine carcinoma (LCNEC) of the lung and small cell lung carcinoma (SCLC) are both now considered as high-grade neuroendocrine carcinomas arising in the lung.[1–4] Based on the large, multiinstitutional study in Japan, Asamura et al.[5] reported that the 5-year survival rates of patients with all stages were 40.3% for LCNEC and 35.7% for SCLC, the differenc not being statistically significant. However, these two tumors are generally thought to have different clinical features[1,4,6–14] and require different treatments.

Currently, surgical resection is advocated for the LCNEC as same as other nonsmall cell lung cancers.[15] However, Iyoda et al.[10] reported that patients with stage I disease treated with either neoadjuvant or postoperative adjuvant chemotherapy had a significantly better prognosis than their counterparts groups receiving surgery alone. Therefore, LCNEC requires a refined histology-specific approach. Conversely, the SCLC is aggressive but chemosensitive, and a standard therapeutic strategy has already been established.[5]

The cytologic diagnosis of SCLC is clear, but criteria for the LCNEC have yet to be established.[5,16–22] Recently, the cytologic features of LCNEC described in several reports are as follows: necrotic background, loose cell aggregates, large cell size (three times as large as mature lymphocytes), rosette and Indian-filing arrangements, abundant cytoplasm, granular nuclear chromatin, clear nucleoli, naked nuclei, and nuclear streaking.[17–22] Because these are also often recognized in SCLC cases,[15,16,23] they are not specific.

The aim of this study was to elucidate the cytologic characteristics of the LCNEC in comparison with SCLCs particularly and evaluate the utility of proposed scoring system for their differential diagnosis.

MATERIALS AND METHODS

Patients

The pathology files of the Cancer Institute Hospital (Tokyo, Japan) between 1990 and 2007 were searched for 29 patients who underwent pulmonary resection for LCNECs.

These LCNEC cases were all confirmed by pathologic examination on surgically resected materials with the World Health Organization (WHO) classification system.[15] The histologic diagnostic criteria of LCNEC proposed by WHO are as follows: neuroendocrine morphologic features (organoid nesting, palisading, rosettes, and trabecular growth pattern); a high mitotic rate (>10 per 10 high-power fields); necrosis (often large zones); cellular features of a nonsmall cell carcinoma (large cell size, a low nuclear/cytoplasmic ratio, polygonal shape, finely granular eosinophilic cytoplasm, coarse chromatin, and/or frequent nucleoli); and neuroendocrine features by immunohistochemistry or electron microscopy or both.[15] For comparison, we randomly extracted 26 cases of SCLCs diagnosed during the same period, 16 of which were diagnosed with surgical materials and the remaining 10 with transbronchial lung biopsy samples. The histologic diagnosis of SCLCs was also based on the WHO classification system.[15] Combined LCNECs and SCLCs and SCLC cases after any treatment were all excluded in this study, which was approved by our institutional review board, each patient giving written informed consent before treatment.

Cytologic Materials

Cytologic specimens obtained by transbronchial aspiration and/or imprint from the resected specimens were fixed routinely in 95% ethanol and stained by the Papanicolaou method. Five to 12 cytologic slides were reviewed for each patient. From previous studies,[17–22] we selected the following parameters for assessment: necrotic background, cellular arrangement, tumor cell clusters, tumor cell cohesion, cell arrangements, cell dimensions areas, the presence of tumor cells with identifiable cytoplasm and/or prominent nucleoli, nuclear features, mitosis, naked nuclei, and nuclear streaking. Cluster size was categorized in the three groups as follows: small clusters, consisting of more than 10 and less than or equal to 20 cells; intermediate-sized clusters, consisting of more than 20 cells and less than 60 cells; and large clusters, consisting of more than or equal to 60 cells. Tight cohesiveness of clusters was defined as a straight cluster border composed of cells lined up and/or arranged in palisades. Cell areas were measured for 50 cells extracted at random in each specimen and calculated as (long diameter + short diameter/$2 \times 2)^2\ \pi$ ($\pi = 3.14$). The diameters of tumor cells were measured using an ocular micrometer (DSM; Olympus, Tokyo, Japan). Cell size was categorized in 2 groups as follows: small tumor cells, less than or equal to 120 μm^2; and large tumor cells, more than or equal to 600 μm^2.

Statistical Analysis

The clinicopathologic factors analyzed in this study included age (<65 or ≥65 years), gender, and smoking habits, evaluated by the χ^2 test. Differences in cell areas and the frequency of the cytologic features between LCNEC and SCLC cases were analyzed by an unpaired Student t test and χ^2 test; $p < 0.05$ was considered significant.

Logistic Discriminant Analysis

To demonstrate the utility of differences in frequencies of cytologic parameters for LCNECs and SCLCs, a discriminant model was developed and evaluated. The frequencies of two cytologic features, in which differences were statistically significant, were regarded as two variables for a set of data, displayed as a scatter plot. By logistic discriminant analysis based on the scatter plots, a discriminant model for LCNEC and SCLC was made. When two variables for frequency of cytologic features were regarded as x_1 and x_2, the probability of an SCLC was calculated as follows.

$$P(SCLC) = \frac{\exp(-319.81 - 10.82x_1 + 16.30x_2)}{1 + \exp(-319.81 - 10.82x_1 + 16.30x_2)}$$

And the discriminant line was as follows.

$$-319.81 - 10.82x_1 + 16.30x_2 = 0 \Leftrightarrow x_2 = 19.62 + 0.6641x_1$$

We regarded a point on upper part of the line as true (SCLCs) and a point on lower part of the line as false (LCNECs). Furthermore, we analyzed prediction of error discrimination for new cases by leave-one-out cross validation. A discriminant model for LCNEC and SCLC was made except in one case. The excepted case was predicted by the discriminant model, and the discrimination confirmed whether it was correct. For all SCLC and LCNEC cases, the same analyses were performed repeatedly.

RESULTS

Clinical Findings

Clinicopathologic findings for the 29 LCNEC patients are summarized in Table 1. There were 26 men and 3 women, ranging in age from 48 to 80 years, with a median of 67 years. Lobectomy was performed on all. Mean follow-up time was 2.4 years (range, 0.33–9 years); 14 were dead, and 15 were alive at the time of this analysis. All patients had a smoking habit, ranging from 3 to 206.5 pack years. Of the 26 SCLC patients, 19 were men and 7 women, ranging in age from 58 to 80 years, with a median of 69 years. Eight were treated with surgical resection and eight with surgical resection after chemotherapy. In these 16 cases, no combination of SCLC with other histologic types was identified on resected materials. The remaining 10 underwent chemotherapy and/or radiotherapy, but again no admixture of other types was noted in biopsy specimens. Mean follow-up for the 26 patients was 2.6 years (range, 0.08–8 years); 14 were dead, and 12 were alive at the time of this analysis. All patients also had a smoking habit. A comparison of data for LCNEC and SCLC groups revealed no statistically significant differences in age, gender, and smoking status (Table 1).

Cytologic Findings

The initial cytologic diagnoses of 29 LCNEC patients were 4 LCNECs, 5 SCLCs, 2 combined SCLCs and adenocarcinomas, 5 neuroendocrine carcinomas, 1 atypical carcinoid, 7 poorly differentiated adenocarcinomas, 3 poorly differentiated squamous cell carcinomas, and 2 nonsmall cell carcinomas. In the LCNEC group, the unanimity in diagnosis between pathology and cytology was 21.1%. The cytologic

TABLE 1. Clinicopathologic Findings for LCNEC and SCLC Cases

Characteristic	No. of Patients	LCNEC (n = 29)	SCLC (n = 26)	*p*
Age (yr)				
<65	14	9	5	0.32
≤65	41	20	21	
Gender				
Male	45	26	19	0.11
Female	10	3	7	
Smoking status				
Nonsmoker	0	0	0	1.00
Smoker	55	29	26	
Cytologic materials				
TBAC	34	16	18	0.56
IC	5	3	2	
TBAC and IC	16	10	6	
Cytologic diagnosis				
LCNEC	4	4	0	<0.0001
LCNEC > SCLC	2	2	0	
NE	4	4	0	
SCLC	31	5	26	
SCLC + NSCLC	2	2	0	
NSCLC	12	12	0	
Tumor location				
Right lung	5	—	5	—
RUL	15	12	3	
RLL	12	7	5	
Left lung	2	—	2	
LUL	14	9	5	
LLL	7	1	6	
Type of location				
Central	11	3	8	0.09
Peripheral	44	26	18	
Tumor size (cm)				
≤3.0	25	12	13	—
>3.0	24	17	7	
NA	6	0	6	
Pathologic stage (pTNM)				
IA	14	8	6	—
IB	12	11	1	
IIA	5	1	4	
IIB	4	3	1	
IIIA	8	4	4	
IIIB	2	1	1	
IV	6	1	5	
NA(LD)	4	—	4	
Survival after surgery				
Dead	28	14	14	0.68
Alive	27	15	12	
Mean ± SD		2.39 ± 2.27	2.63 ± 2.33	

TBAC, transbronchial aspiration cytology; IC, imprint cytology; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; NE, neuroendocrine carcinoma; NSLC, nonsmall cell lung carcinoma; RUL, right upper lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; NA, not available; pTNM, from Ref. 15; LD, Limited disease.

TABLE 2 Cytologic Comparison Between LCNEC and SCLC

Cytologic Parameters	LCNEC (n = 29)	SCLC (n = 26)	*p* value
Necrotic background	25/29 (86.2%)	23/26 (88.5%)	0.802
Predominant cellular arrangement			
Cluster	26/29 (89.7%)	5/26 (19.2%)	
Single cells	3/29 (10.3%)	21/26 (80.7%)	<0.0001
Presence of characteristic clusters			
Large sized	27/29 (93.1%)	4/26 (15.4%)	<0.0001
Strong cohesion	27/29 (93.1%)	3/26 (11.5%)	<0.0001
Presence of tumor cell arrangement			
Rosette	28/29 (96.6%)	21/26 (80.7%)	0.061
Molding	26/29 (89.7%)	26/26 (100 %)	0.092
Pair cells	12/29 (41.3%)	17/26 (65.4%)	0.075
Palisading	27/29 (93.1%)	3/26 (11.5%)	<0.0001
Mean tumor cell size	178.1 μm^2	127.2 μm^2	<0.0001
Presence of characteristic tumor cells			
Large sized	18/29 (62.0%)	20/26 (76.9%)	0.224
Evidently identifiable cytoplasm	27/29 (93.1%)	20/26 (76.9%)	0.089
Prominent nucleoli	24/29 (82.8%)	20/26 (76.9%)	0.589
Small sized without prominent nucleoli	15/29 (51.7%)	26/26 (100 %)	<0.0001
Chromation pattern			
Finely granular	10/29 (34.5%)	11/26 (42.3%)	
Finely granular to granular	14/29 (48.3%)	15/26 (57.7%)	0.085
Granular	5/29 (17.2%)	0/26 (0 %)	
Presence of characteristics			
Mitoses	25/29 (86.2%)	25/26 (96.2%)	0.200
Nuclear streaking	26/29 (89.7%)	25/26 (96.2%)	0.354
Naked nuclei	24/29 (82.8%)	10/26 (38.5%)	0.0007

diagnoses for the 26 SCLC patients were all SCLCs, with statistically significant unanimity ($p < 0.0001$).

In a preliminary study, we evaluated any cytologic differences between aspiration smears and touch preparations in pilot groups consisting of 10 cases each of LCNEC and SCLC. In these groups, aspiration preparations and imprints showed no significant differences in any of the parameters chosen for assessment (data not shown). Comparisons between LCNEC and SCLC for each cytologic parameter are shown in Table 2. Cytologic parameters with statistically significant differences were as follows: cellular arrangement, presence of large clusters, tumor cell cohesion, palisading arrangement of tumor cells, mean of cellular areas, and presence of small cells without prominent nucleoli and naked nuclei. With regard to cellular arrangement, single cells were evident in all SCLC cases, whereas tumor cell clusters were frequently observed in LCNECs (Figures 1, 2). In the LCNEC group, although single cells were evident, many of them had naked nuclei. In particular, large clusters consisting of more than 60 cells were characteristic in LCNEC group

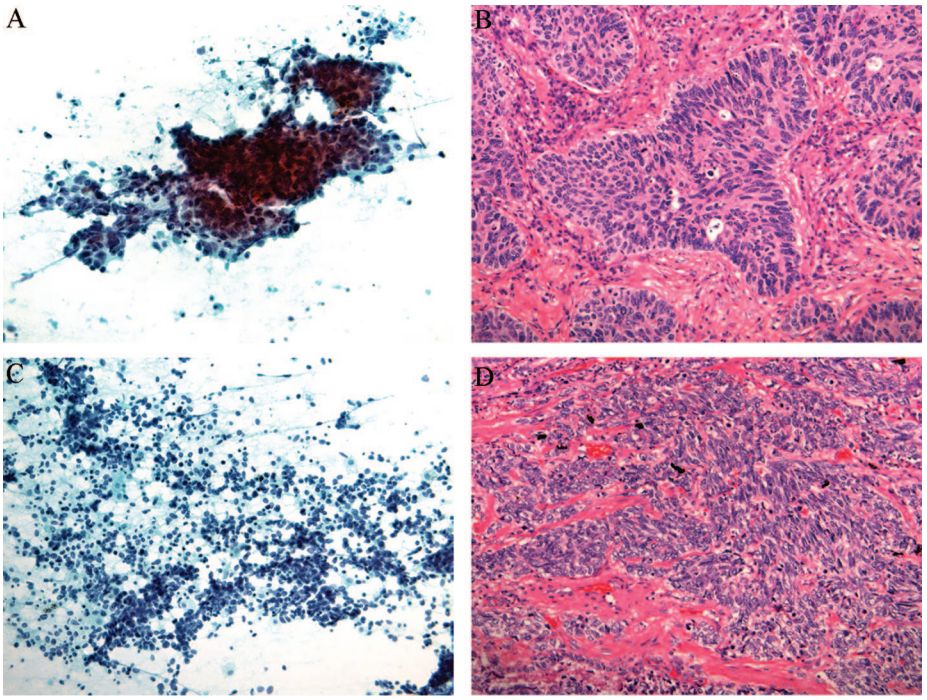

FIGURE 1. Photomicrographs illustrating cellular arrangement in transbronchial aspiration or histology specimens of LCNEC and SCLC cases. *A,* Large and three-dimensional clusters are conspicuous in a cytologic smear of an LCNEC case (Papanicolaou stain, ×20); *B,* tumor nests of an LCNEC case show palisading and Rosette-like formations in a histology specimen (hematoxylin and eosin stain, ×40); *C,* single cells are conspicuous in the cytologic smear of an SCLC case (Papanicolaou stain, ×20); and *D,* tumor cells of an SCLC case comprise irregular nests in a histology specimen (hematoxylin and eosin stain, ×40).

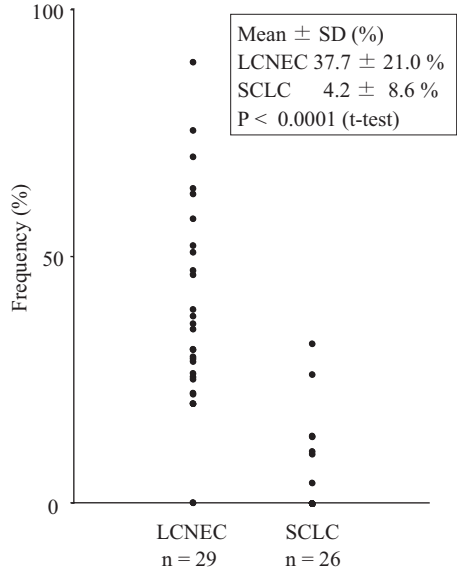

FIGURE 2. Frequencies of large clusters with tight cohesion in the LCNEC and SCLC groups (n = 55).

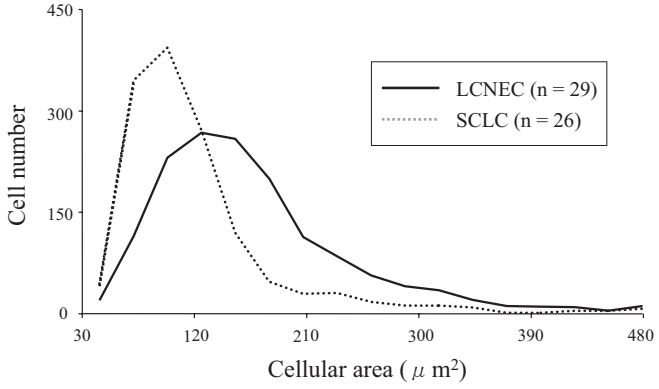

FIGURE 3. Histograms of cell areas in LCNEC and SCLC groups. Note that about 60% of the SCLC cells fall in the range of less than 120 μm^2, as compared with about 25% for LCNEC cells ($p < 0.0001$).

(Figures 1, 2). Also, on those histologic specimens, cell adhesion between tumor cells of LCNEC cases was conspicuous, whereas it was indistinct in SCLC cases (Figures 1*B*, *D*).

Furthermore, tumor cell cohesion was weak in SCLC cases, whereas in LCNEC cases tightly cohesive clusters predominated (Table 2). Frequencies of large clusters with tight cohesion are shown in Figure 3. The mean frequency was 37.7 ± 21.0% in the LCNEC cases and 4.2 ± 8.4% in SCLCs, the difference being statistically significant ($p < 0.0001$). LCNEC cases featured discrete cell nests divided by fibrous stroma with frequent peripheral palisading, whereas SCLC cases were characterized by cell nests, frequently infiltrating adjacent fibrous stroma (Figure 1).

Mean cell areas were 178.1 ± 84.8 μm^2 (range, 45.3–808.9 μm^2) for LCNEC cases and 127.8 ± 69.3 μm^2 (range, 36.8–699.5 μm^2) for SCLCs, the difference being statistically significant ($p < 0.0001$). The distributions are shown graphically in Figure 3. Some 58.2% of the SCLC cells (756/1300) were less than 120 μm^2, as compared with only 24.6% for LCNEC cells (357/1450; $p < 0.0001$). Furthermore, small tumor cells lacking prominent nucleoli in SCLC cases were observed more frequently than in LCNEC cases ($p < 0.0001$; Table 2 and Figure 5). Frequencies are shown in Figure 4. The mean values were 11.9 ± 12.1% in LCNEC and 55.8 ± 18.9% in SCLC cases, the difference being

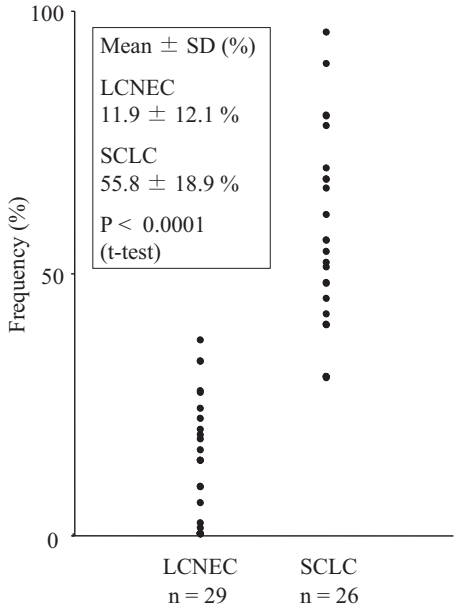

FIGURE 4. Frequencies of small tumor cells without prominent nucleoli in the LCNEC and SCLC groups (n = 55).

statistically significant ($p < 0.0001$). Also, in histologic specimens, SCLC cases had the cell nests predominantly composed of small tumor cells with scant cytoplasm without nucleoli, whereas LCNEC cases demonstrated cell nests predominantly composed of large tumor cells with abundant cytoplasm and occasional prominent nucleoli (Figure 5*B*, *D*).

Logistic Discriminant Analysis

For the frequencies of large clusters with tight cohesion and small tumor cells without prominent nucleoli, statistically significant differences with strong power was evident between LCNEC and SCLC groups. Therefore, these two cytologic parameters were considered as the two variables for the scatter plots. The dots for LCNEC cases are located on the lower right, whereas those of SCLC cases were located on the upper left, with clear differences between the two for the majority. The results of logistic discriminant analysis are shown in Figure 6. Because all SCLC and LCNEC cases were cytologically discriminated accurately, sensitivity, specificity, and accuracy were all 100%. Moreover, the results of leave-one-out cross validation, shown in Figure 7, gave a predicted error rate of $(2 + 1)/55 = 0.00545$.

DISCUSSION

In this study, the large cell cluster with tight cohesion was confirmed to be a valuable cytologic feature, allowing distinction between LCNECs and SCLCs. Although other reports on cytologic features of LCNECs described that cell cohesion of LCNECs was reduced as in SCLC,[17–22] the difference was highly significant in our series. However, palisade arrangement was described as a one point for cytologic distinction of LCNEC from SCLC,[17–22] and it was considered to be easy to detect tight cell cohesion by light microscope. Therefore, it should be emphasized that focusing on large clusters with tight cohesion is most important for cytologic discrimination between LCNECs and SCLCs.

Several authors showed that tumor cells of LCNECs had similar morphologic features to SCLCs except for cell size, this being significantly larger for LCNECs than SCLCs.[17–22] In these series, a majority of the SCLC cells were less than 120 μm^2 in size, statistically significant as compared with LCNEC cells ($p < 0.0001$). Another characteristic was that most of small cells in SCLC cases had no

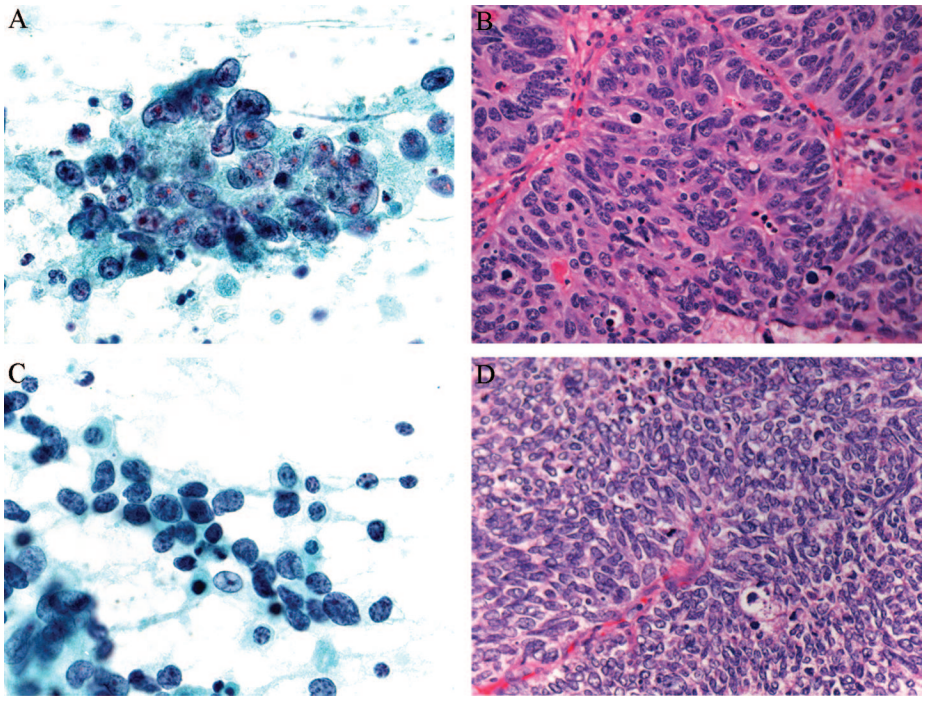

FIGURE 5. Photomicrographs illustrating single cells and tissue architecture in LCNEC and SCLC cases. *A*) Tumor cells ≥120 μm^2 and/or with prominent nucleoli are evident in a cytologic smear of an LCNEC case (Papanicolaou stain, ×100); *B*, nests of LCNEC cells are predominantly composed of large tumor cells with abundant cytoplasm and occasional prominent nucleoli (hematoxylin and eosin stain, ×40); *C*, tumor cells <120 μm^2 without prominent nucleoli are evident in a cytologic smear of an SCLC case (Papanicolaou stain, ×100); *D*, nests of SCLC cells are predominantly composed of small tumor cells with scant cytoplasm without nucleoli (hematoxylin and eosin stain, ×40).

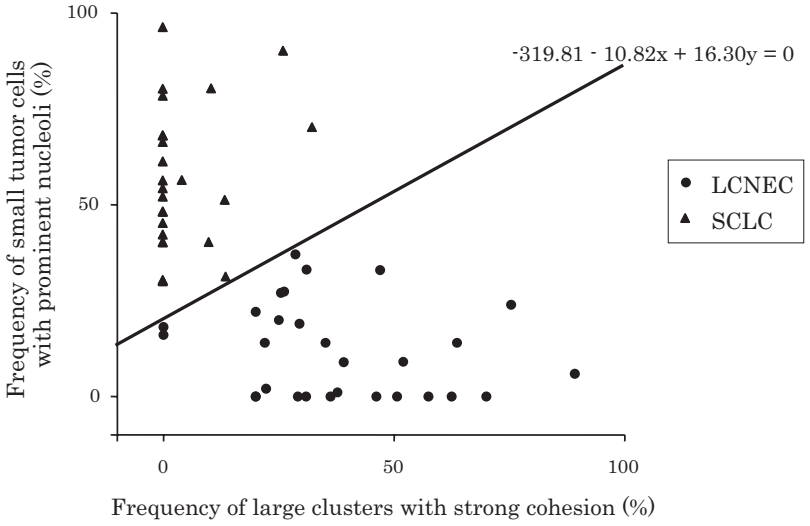

FIGURE 6. Logistic regression for frequencies of small tumor cells without prominent nucleoli and large clusters with tight cohesion. The scatter plot allows clear separation of LCNEC (●) and SCLC cases (▲) by the calculated discriminant line. Therefore, a discriminant model for LCNEC and SCLC, diagnosing as SCLCs if dots exist in the field above the line and as LCNECs if below the line, was made. All SCLC and LCNEC cases were discriminated correctly with the discriminant model based on logistic regression from cytologic frequencies, and sensitivity, specificity, and accuracy were all 100%.

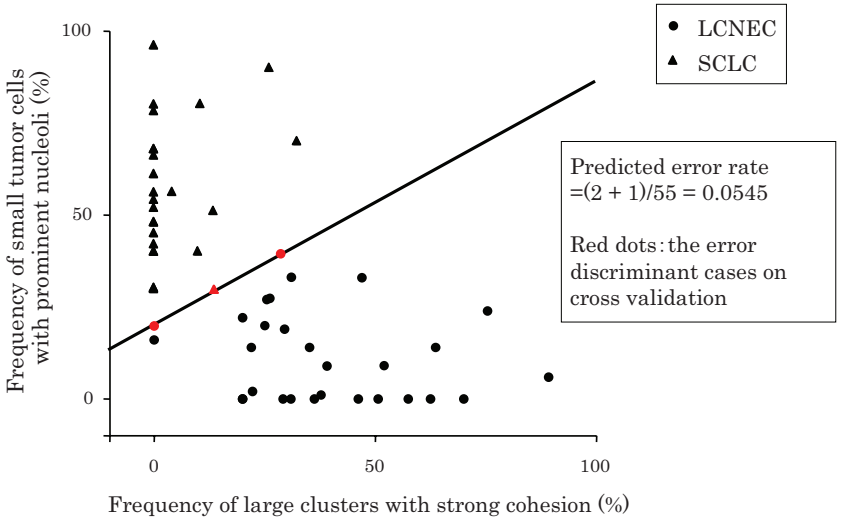

FIGURE 7. Leave-one-out cross validation. Regarding each case as a new case, prediction of the error rate of the discriminant model was analyzed. Red dots show error discriminant cases on cross validation. Two of the LCNEC cases and one SCLC case were discriminated in error with the discriminant model, so that the prediction of error rate of the model was 0.00545.

prominent nucleoli, again being significantly different from LCNECs ($p < 0.0001$). Moreover, although naked nuclei appear to be a significant distinguishing attribute between the two tumor types, it was considered to be inadequate for inclusion in the discriminant model for the following reasons: it is rather difficult to perceive cytoplasm in intact large cells compared with small cells, and naked nuclei was not found in more than 60% of SCLC cases. Therefore, only the frequency of the small cells without prominent nucleoli contributed to cytologic discrimination between LCNEC and SCLC.

To establish accurate cytologic diagnosis of LCNEC using the two cytologic parameters, we established a discriminant model that gave exceedingly good sensitivity, specificity, and accuracy. The current discriminant model, however, does have some problems with routine cytology as follows: complicated procedures for obtaining the two cytologic parameters and necessity of uniform diagnostic criteria among cytopathologists. However, with greater experience of LCNEC cases and grasp of detailed cytologic features, it should be possible to overcome these problems.

In conclusion, our discriminant model based on the cytologic features of large cell clusters with tight cohesion and of small tumor cells without prominent nucleoli should prove a useful aid for distinction between LCNECs and SCLCs particularly. Prospective large-sized studies including other nonsmall cell lung cancers are now required to assess the diagnostic impact of this model with routine cytology.

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